16.

Studies on Virus Diseases of Fish.
IV. Lymphocystis Disease in Centrarchidae.

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(Plates I—III).

INTRODUCTION.

The lymphocystis virus disease of fish is prominent among other virus diseases by the fact that the infected cells represented by fibroblasts and osteoblasts undergo a gigantic hypertrophy. By a characteristic metamorphosis the host cells are transformed into the so-called lymphocystis cells. They contain cytoplasmic inclusion bodies which at early stages resemble in their configuration those observed in certain other virus diseases (mammalian pox group for instance). In connection with the conspicuous hypertrophy of the host cell, however, the inclusion bodies in the lymphocystis disease grow to enormous dimensions, unique hitherto in the pathology of virus diseases.

The lymphocystis disease of fish first studied in Europe has been observed among American fish since 1936 in several marine species of the Atlantic coast of the United States (blue angelfish, *Angelichthys isabelita* [Smith and Nigrelli, 1937], hogfish, *Lachnolaimus maximus* [Weissenberg, Nigrelli, Smith, 1937], orange filefish, *Aleutera schoepfii=Ceratacanthus schoepfii*, [Weissenberg, 1938; Nigrelli and Smith, 1939], common killifish, *Fundulus heteroclitus*, [Weissenberg, 1939a]). Among North American fresh water fish the occurrence of lymphocystis disease has been described hitherto only in the perch *Stizostedion*. It has been studied especially in *Stizostedion vitreum*, the so-called wall-eyed pike perch (cf. Mavor and Feinberg, 1918; Hyde, 1937; Weissenberg, 1939b).

Since 1938 I had the opportunity to ascertain the occurrence of lymphocystis disease in several species of the Centrarchidae or sunfish which are known to represent a large and significant family of fresh water fish peculiar to North America.

The first information that lymphocystis disease sometimes occurs in Centrarchidae was given to me by Dr. R. R. Kudo, University of Illinois, Urbana, Ill., in 1937, soon after my arrival in the United States. Dr. Kudo wrote me concerning the lymphocystis disease of *Stizostedion* and incidentally mentioned that in the preceding years he had observed lymphocystis tumors in specimens of the white crappie, the black crappie and the bluegill taken in Illinois. Dr. Kudo has not published his observations and was so kind as to send me in 1939 some preserved material of affected Centrarchidae for my comparative study. In the meantime I had discovered and observed a lymphocystis epidemic of bluegills and common sunfish at the Aquarium of Philadelphia in the winter of 1938-1939. These fish were taken from the Schuylkill River in Philadelphia. I am greatly indebted to Dr. Robert O. Van Deusen, Director of the Philadelphia Aquarium, for placing infected specimens at my disposal. In that winter I had excellent facilities for keeping sunfish and bluegills in laboratory aquaria at the E. B. Morris Biological Farm of the Wistar Institute of Anatomy and Biology, Bristol, Pa. The epidemiological and experimental observations on these Centrarchidae are a part of my researches done at the E. B. Morris Biological Farm.

In the following winter I observed again at the Philadelphia Aquarium a lymphocystis epidemic of sunfish and bluegills. Furthermore, I saw in 1939, 1940 and 1941 several cases of lymphocystis disease in other species of Centrarchidae, partly collected also from the Schuylkill River in Pennsylvania, partly from other regions of the United States. For providing material of lymphocystis-affected Centrarchidae from Ohio, Michigan, Missouri and New Mexico, I am very grateful to Dr. R. V. Bangham, Wooster College, Wooster, Ohio, and to Dr. C. L. Hubbs, then at Univer. of Michigan, Museum of Zoology, Ann Arbor, Mich.

PROPAGATION OF LYMPHOCYSTIS DISEASE AMONG CENTRARCHIDAE.

The present study of the lymphocystis disease of the Centrarchidae is chiefly based on the numerous infected specimens of *Lepomis gibbosus* (Linnaeus), common sunfish, and *Lepomis macrochirus* Rafinesque, bluegill, in which I was able to follow the

T A B L E I.

Material of lymphocystis diseased Centrarchidae (Genus *Lepomis*)

Species	Places of Collection	Epidemics	Speci-mens Studied In Detail	First In-spected By	Length Of Spec-imens	Dates Of Collec-tion	Greatest Size Of Lympho-cystis Cells	Young Lympho-cystis Cells (Reinfec-tion)
Lepomis gibbosus	Schuylkill River at Philadelphia, Pennsylvania	Philadelphia Aquarium Dec. to May a) 1938/39 b) 1939/40	18	Weissen-berg	8-15 cm.		601 × 515 micra	observed in several specimens
Lepomis macro-chirus	Schuylkill River at Philadelphia	Philadelphia Aquarium cf. above	20	Weissen-berg	8-20 cm.		669 × 624 micra	observed in several specimens
	Illinois		1	Kudo			440 micra	missing
Hybrid Lepomis cyan-ellus × Lepo-mis macro-chirus	Dexter, New Mexico	U. S. Fish Serv. Hatchery Dexter, New Mex. in winter 1940/41	1	Hubbs		Dec. 19, 1940	300 micra	missing
Lepomis mega-lotis	St. Francis River near Greenville, Missouri		1	Hubbs		July 11, 1941	170 micra	missing

course of the disease on the living fish in Philadelphia. Further by study of individual cases the occurrence of the disease has been ascertained in the following species[1]: 1) hybrid, *Lepomis cyanellus* Rafinesque × *Lepomis macrochirus* Rafinesque. 2) *Lepomis megalotis* Cope, long-eared sunfish. 3) *Pomoxis nigro-maculatus* (LeSueur), black crappie or calico bass. 4) *Huro salmoides* (Lacépède), large-mouthed bass. 5) *Micropterus pseudaplites* Hubbs, Kentucky bass or spotted bass.

Thus lymphocystis disease has been ascertained in six species of Centrarchidae and in a hybrid of which the male parent represents a seventh, different species. To the host list may be further added *Pomoxis annularis* Rafinesque, the white crappie, in which species Dr. Kudo observed lymphocystis-infected specimens in Illinois (personal communication). The eight members of the Centrarchidae which have thus been ascertained as occasional hosts of lymphocystis disease belong to four different genera, namely *Huro*, *Lepomis*, *Micropterus* and *Pomoxis*. Therefore, it can be stated that susceptibility to lymphocystis disease is indeed widespread in the Centrarchidae.

Tables I and II give more detailed information about the material studied. From column 2 it can be seen that the infected specimens were taken from waters of six different states (Illinois, Michigan, Missouri, New Mexico, Ohio, Pennsylvania).

[1]The nomenclature follows mainly Hubbs and Lagler, 1939.

Therefore, it can be stated that lymphocystis disease is without any doubt widely spread in the United States. The disease seems to occur rather frequently, at least in *Huro salmoides* and some of the species of *Lepomis*.

Several of the Centrarchidae listed play an important role not only as game fish but also as food fish. Therefore, the study of this disease is not only of scientific interest but is also of some economic significance. I am referring, for instance, to the epidemic in hybrids of *Lepomis cyanellus × macrochirus* observed in the Hatchery of the U. S. Fish and Wildlife Service at Dexter, New Mexico. Likewise, it is of interest to note that many young specimens of the large-mouthed bass were affected in a lake in Ohio. Furthermore, the outbreaks of lymphocystis disease among the common sunfish and the bluegills which I observed at the Philadelphia Aquarium involved numerous specimens.

Concerning the season of the occurrence of the disease, column 3 of Table I shows that the lymphocystis disease of *Lepomis gibbosus* and *macrochirus*, observed as epidemics at Philadelphia in two successive years, was restricted to the cold weather period. The outbreak of lymphocystis disease in the hybrids at the U. S. Fish Hatchery at Dexter, New Mexico, likewise occurred during the winter season.

On the other hand there are observations of the occurrence of lymphocystis disease in other species of Centrarchidae in the sum-

TABLE II.

Material of lymphocystis diseased Centrarchidae (Genera *Pomoxis, Huro, Micropterus*)

Species	Places of Collection	Epidemics	Speci-mens Studied In Detail	First in-spected By	Length of Spec-imens	Dates Of Collec-tion	Greatest Size of Lympho-cystis Cells	Young Lymphocys-tis Cells (Reinfec-tion)
Pomoxis nigro-macu-latus	Illinois		1	Kudo			380 micra	missing
	Schuylkill River at Philadelphia, Pennsyl-vania		1	Weissen-berg	7 cm.	Nov. 1939	148 × 103 micra	missing
Huro sal-moides	Half Moon Pond north of St. Mary's Ohio	numerous young specimens diseased	1	Bang-ham	7 cm.	July 19, 1939	300 micra	observed in sizes up to 81 micra
	Schuylkill River at Philadelphia		1	Weissen-berg	13.5 cm.	Sept. 15, 1939	535 micra	observed in sizes up to 117 micra
	Huron River mouth of Silver Creek Wayne Co., Michigan		1	Hubbs	13.2 cm.	Oct. 12, 1941	330 micra	observed in sizes up to 150 micra
Microp-terus pseuda-plites	Wheeler Reservoir Ohio		1	Bang-ham	"adult"	Sept. 26, 1938	300 micra	missing

mer time. A rather young lymphocystis infection was seen in a specimen of *Lepomis megalotis* taken and preserved in the first half of July. It was also in the summer season that the frequent occurrence of lymphocystis disease in young large-mouthed bass was observed in a pond in Ohio. The only infected specimen of this species which I secured at the Philadelphia Aquarium had been taken from the Schuylkill River about the middle of September, which is still in the warm weather period for Philadelphia. It is of interest to note that this was at least seven weeks before bluegills and common sunfish taken at the same locality showed any macroscopic signs of lymphocystis disease.

Concerning the maximal diameters of lymphocystis cells observed in the collected Centrarchidae and listed in Tables I and II, it should be understood that only in *Lepomis gibbosus* and *macrochirus* the diameters in column 8 represent the greatest size of full-grown lymphocystis cells as observed in material from several specimens. Concerning all the other species listed, the diameters in column 8 refer to lymphocystis cells of the recorded specimen only and do not represent measurements of full-grown cells.

It is of interest to compare the maximal diameters observed in the full-grown lymphocystis cells of the common sunfish (601 microns) and the bluegill (669 microns) with those stated in other fish groups. In reference to the largest dimensions of lymphocystis cells hitherto described, the two Centrarchidae are intermediate in position between *Lachnolaimus* and *Acerina*. In *Lachnolaimus* tumors, lymphocystis cells of 530 microns were observed. In *Acerina* the lymphocystis cells reach 700 microns. In comparison with full-grown lymphocystis cells of *Stizostedion* (1,200 microns) and *Pleuronectes* (2,000 microns), the lymphocystis cells of the two Centrarchidae only reach medium dimensions at the end of their growth period.

COURSE OF THE LYMPHOCYSTIS DISEASE IN *Lepomis gibbosus* AND *macrochirus*. SOME INFECTION EXPERIMENTS.

The general aspect of the lymphocystis tumors in the Centrarchidae is similar in appearance to that of the lymphocystis disease in other fish groups. Round nodules or oblong growths two to three millimeters in length protrude from the skin. The growths are covered by the epidermis and by a thin layer of connective tissue containing pigment cells. The chief component of the growths is represented by the hypertrophied and transformed fibroblasts, the so-called lymphocystis cells. When inspected through the magnifying glass or in later stages with the naked eye, these cells appear more or less distinctly as round bodies shining through the covering layers.

The lymphocystis tumors of the Centrar-

chidae when compared with those of the perch *Stizostedion* show the following peculiarities. 1) The tumors are, as a rule, restricted to the fin borders. Very seldom the skin of the trunk is affected. 2) Usually only a few tumors develop, which in the average consist of less lymphocystis cells than in the perch. Sometimes only a very small number of fibroblasts or osteoblasts of a fin become transformed into scattered lymphocystis cells. 3) The color of the tumors in *Lepomis gibbosus* and *macrochirus* is not much modified by covering pigment cells. Tumors in advanced stages of growth appear white because the lymphocystis cells of the Centrarchidae become white and opaque as soon as they have reached macroscopic dimensions. Therefore, rather full-grown tumors are clearly visible on the swimming fish even at a distance and growths which contain only a few lymphocystis cells can be recognized as white dots.

Previous observations on *Acerina cernua* and *Pleuronectes flesus* had shown that lymphocystis disease is very infectious for these fish when kept together with diseased specimens of their own species (Weissenberg, 1914, 1921b). The observations on the common sunfish and bluegills in the large tanks of the Philadelphia Aquarium as well as in the laboratory aquaria confirmed this experience, at least in principle. It is true that not always under these conditions did infection occur, but about 60 percent. of healthy fishes became infected when living in the same container with diseased specimens of the same species. After several weeks they developed lymphocystis tumors, or at least some lymphocystis cells which after reaching macroscopic size became clearly visible as white spots.

The epidemic discovered in the Philadelphia Aquarium in December, 1938, among specimens taken from the Schuylkill River, came to its end during the spring. In May the last carriers of tumors became clean by sloughing. These observations corresponded with the results in the laboratory. From May on, infection experiments no longer gave any positive results. So it seems that the susceptibility of the common sunfish and the bluegills persisted only during the cool weather period.

For experimental infection I applied with success the two methods which I developed in previous experiments with perches and *Pleuronectes*. An emulsion of the tumors was either put into the aquarium water or it was sprayed into the pharynx of specimens through the mouth or through the opercular clefts.

Most efficient was an emulsion of tumor material which was very finely comminuted, first by cutting and teasing the tumors, and then by grinding them thoroughly in a mortar. The tumors used in preparing this emulsion were not emulsified immediately after their excision but were first placed in the refrigerator for 24 hours. Further, the emulsion itself was kept in the laboratory for several hours before it was applied to the fishes. This procedure was followed with the idea in mind that the efficiency of the material for infection might be increased by changes going on within the lymphocystis cells after the excision of the tumors.

In contrast to the successful infection results obtained with tumor emulsions it is of interest to note that the feeding of whole pieces of sunfish tumors to three sunfish did not produce infection.

On specimens of *Lepomis gibbosus* and *macrochirus* kept in laboratory aquaria I observed that the whole course of the disease took only 16-18 weeks. The macroscopic manifestation of the lymphocystis disease in the common sunfish was the same as in the bluegill. The whole course of the disease can be described for these two Centrarchidae with regard to macroscopic observation as follows: After infection about six weeks elapsed until the tumors became conspicuous to the naked eye as gray transparent swellings. After another week they had increased in size and began to appear whitish. During the following weeks the tumors, now definitely white, continued to grow. The full grown stage was reached approximately twelve weeks after infection. Two weeks later the tumors seemed to be still intact but after another two weeks they looked considerably smaller as a result of shedding of numerous lymphocystis cells or of sloughing off of whole pieces of tumors. The process of shedding was finished about two weeks later. Thus, the whole process occurred in sixteen to eighteen weeks. This statement applies to common sunfish and bluegills which were kept from December to June in laboratory aquaria without running water. The rooms in which the aquaria were situated were moderately heated during the winter.

The observation that the skin of the diseased Centrarchidae became clean again soon after the termination of the growth of the tumors is in a certain contrast to my previous experience with *Acerina*. At least a few of the infected specimens of this perch did **not** slough off their tumors but carried them still seven weeks after the expiration of the growth period of the lymphocystis cells. About that time an intercurrent *Saprolegnia* infection terminated their life in the laboratory aquarium. The persistent tumors consisted mainly of degenerated lymphocystis cells. The observation of the healing of the lymphocystis disease in the two Centrarchidae is, on the other hand, in complete accordance with the observations of Nigrelli and Smith on the filefish. It may be that in *Acerina* also the percentage of infected fish

showing final loss of their tumors would have been larger if it had been possible to keep them for a longer period of observation.

The statement that the lymphocystis disease in the two Centrarchidae healed, as a rule, two weeks after the end of the growth period of the lymphocystis cells refers to the macroscopic appearance of the fish. I do not doubt that a microscopic examination would still be able to demonstrate traces of the recent disease. For instance the resistant membranes of degenerated lymphocystis cells may persist for some time or some stunted cells may be found which never reached macroscopic dimensions due to arrested development at an early stage.

Persistence of such small lymphocystis cells from a previous infection can, of course, become a source of error in judging results of infection experiments. There is the danger of confusing such arrested cells with young lymphocystis cells produced by the experiment at hand. Because in the experiments with the two centrarchids the fish chosen for the experimental treatment were from the same river from which carriers of tumors were taken, great care was necessary to avoid possible sources of error.

A confusion of arrested cells of a previous infection with young lymphocystis cells could, however, be excluded by the following procedure which is based on the fact that the borders of the fins represent a favorite place for the development of lymphocystis cells. Three weeks before the start of the infection experiment the border zone of the tail fin and the anal fin of the specimens to be experimented with was amputated. When the infection experiment started the regeneration of the fins had already progressed well. Three weeks later the fin borders were amputated again and examined microscopically. Should they now contain small lymphocystis cells within the regenerated zone, then it was evident that these lymphocystis cells were young lymphocystis cells developed during the period of the experiment, and could not be any arrested remnants from an old infection.

Plate III, Fig. 11, from a positive infection experiment, shows as an example of such a preparation a whole mount of the border region of the tail fin of a bluegill. The epithelium has been removed to a large extent with a brush. The upper part of the figure shows the regenerated zone (**re**) which can easily be recognized by the dark staining of the tissue still in differentiation. New end portions of the fin rays already can be seen (**rf**), but bony plates are not yet developed in them, in contrast to the clearly-visible bony plates (**bo**) of the old fin rays in the lower part of the figure. Some debris of bone plates (**d**) show dis-

tinctly the level at which the amputation was performed. The pigment cells (**p**) are larger in the nonregenerated zone. The preparation shows very clearly small lymphocystis cells (**l**). Some of them lie isolated, some form small groups. In the middle of the preparation they are assembled in a large cluster. The important fact is that all these small lymphocystis cells are developed exclusively in the regenerated zone. Here they lie between or on the regenerating fin rays. Thus this preparation gives the clear evidence for the positive result of such an infection experiment.

Specimens of *Lepomis gibbosus* and *macrochirus* and also of some other species of the collected Centrarchidae with tumors containing large lymphocystis cells frequently showed in the tumors also young lymphocystis cells representing the manifestation of a second attack of the disease (cf. Tables I and II, column 9). Common sunfish and bluegills still bearing tumors of a previous infection could be experimentally re-infected by administration of tumor emulsion. In some specimens three subsequent lymphocystis infections were seen during four months. In contrast to observations made on *Acerina* (Weissenberg, 1921b, p. 1367), it was observed in the two centrarchids that a second infection might follow a first one after only a few weeks.

Experiments in which I tried to infect common sunfish or bluegills with an emulsion of *Stizostedion* lymphocystis cells were unsuccessful. This result corresponds with the negative result of previous experiments in which I attempted to transmit lymphocystis disease from *Stizostedion* to *Fundulus heteroclitus* and *diaphanus* (Weissenberg, 1939a). That experience has led me to the conclusion that different kinds of lymphocystis viruses have to be distinguished which are adapted to different fishes (Weissenberg, 1939a, p. 255).

In considering the question as to how strictly the virus strains may be adapted to their hosts it must, of course, be kept in mind that *Stizostedion* and *Fundulus,* and also *Stizostedion* and *Lepomis,* belong to separate families which are more or less remote in their systematic relationship. Experiments in transmitting lymphocystis disease from one species to another species of the same genus had not hitherto been performed. The simultaneous occurrence of lymphocystis disease in the two species of *Lepomis* at the same locality now raised the question as to whether the epidemics of the sunfish and bluegills were produced by the same type of virus, or if here also two different strains have to be distinguished. This problem was approached by the following experiment. An emulsion of full-grown tumors of a bluegill, prepared as described above, was sprayed into the pharynx of

three bluegills and two sunfish. Twenty days later all these five fishes showed in their fins young lymphocystis cells at the same stage of development. The infection of one sunfish was especially severe. None of the two sunfish carried any remnants of lymphocystis cells from a previous infection which could have been the source for a re-infection. Thus the referred experiment indicates that the bluegill virus is transmissible to the common sunfish.

Unfortunately the season was too advanced to start any more similar experiments, and in the following winter I no longer had the facilities at my disposal for a continuation of the experimental work. As far as a conclusion may be derived from only one experiment, the referred observation is in favor of the presumption that the simultaneous epidemics among the two species of *Lepomis* in the Schuylkill River were caused by the same agent.

MICROSCOPIC STRUCTURE OF THE LYMPHO-CYSTIS CELLS. THE CYTOPLASMIC INCLU-SION BODIES IN SEVERAL STAGES OF DEVELOPMENT.

The lymphocystis cells of the Centrarchidae show, in comparison with those of other fish groups, some morphological peculiarities. However among the various host species within this family, there are not significant differences, on the basis of my present knowledge. Especially for the lymphocystis cells of the common sunfish and the bluegill, of which I have seen the most material, is it true that they are not essentially different in their structure.

Corresponding with the findings in other fish groups the lymphocystis cells of the Centrarchidae have a spherical or oval shape and are surrounded by a glassy homogeneous membrane which shows a strong index of refraction. In fresh preparations the membrane represents a thick envelope; however, it shrinks during fixation, especially in younger cells. Therefore, in Pl. I, Figs. 1-3, 6, 7, the membrane (m) appears only as a single contour. The membrane shows a basophilic staining reaction, but like mucin or the ground substance of cartilage it does not keep the basic staining so firmly as the chromatin does.

The lymphocystis cell contains a large vesiculated nucleus which is rather poor in chromatin content especially in advanced stages of the growth period. Occasionally binucleated lymphocystis cells are found as in other fish groups. With regard to the number of acidophil nucleoli the lymphocystis cells of the Centrarchidae resemble those of the perches. Usually the nucleus contains a single nucleolus as can be seen in most figures of Pl. I and in Pl. III, Fig. 10. By exception two nucleoli are found (Pl. II, Fig. 9).

The most conspicuous structures within the cytoplasm of the lymphocystis cells are their inclusion bodies which grow rapidly within the hypertrophying cells. Their configuration shows some differences in the lymphocystis cells of various fish groups. It seems, however, that they always pass through a stage in which they resemble very much the Guarnieri bodies which appear in the cells of mammals infected by vaccinia virus. I have always laid great stress upon the demonstration of this stage of development because it so clearly illustrates the similarity of the lymphocystis inclusion bodies to characteristic features known in other virus diseases (mammalian pox group). Pl. I, Figs. 1-3, show the "Guarnieri body stage" of the inclusions in bluegill lymphocystis cells and Fig. 4 in a lymphocystis cell of the common sunfish. The inclusions are in this stage represented by small round or oval bodies which are surrounded by a halo.

In respect to the number of the inclusion bodies which attain full development, the lymphocystis cells of the different fish groups represent two types. In the first type normally only one inclusion body sprouts out and additional inclusion bodies usually remain as rudiments. This type is represented by the lymphocystis cells of the perches (*Acerina, Stizostedion*). The lymphocystis cells of *Macropodus, Sargus* and *Aleutera* (filefish) belong likewise to this type. In the second type numerous inclusions develop into larger bodies. This type is represented by the lymphocystis cells of the Pleuronectidae and of *Lachnolaimus*.

The lymphocystis cells of the common sunfish and the bluegill belong to type I. Usually the young lymphocystis cells contain only one inclusion body, as Fig. 1 demonstrates for the bluegill and Fig. 4 for the common sunfish. In some lymphocystis cells additional inclusion bodies are found; as a rule, however, these remain rudimentary. In Fig. 2 (bluegill cell) there is to be seen in addition to the right inclusion body which has already grown larger an accessory small inclusion on the left side. Fig. 3 shows, likewise in a bluegill lymphocystis cell, the rare case of two accessory inclusions.

In the perches (*Acerina, Stizostedion*) the young inclusion body then takes the shape of a fenestrated calotte which lies to one side of the nucleus. As Fig. 5 demonstrates in a lymphocystis cell of *Lepomis gibbosus*, also in the Centrarchidae sometimes the expanding inclusion body develops as a calotte. Usually, however, the growth of the inclusion body progresses mainly in the longitudinal dimension, so that the body forms a long cord which embraces the nucleus. Fig. 7 shows this in a sunfish lymphocystis cell which is cut in

two sections (A and B). Fig. 6 demonstrates the same in a bluegill cell. The portion of the inclusion cord situated beneath the nucleus is shown in the drawing in lighter color. A modification, in which the growth of the inclusion body extended predominantly in the longitudinal dimension but also to some degree in the transverse, is represented by a sunfish lymphocystis cell in Fig. 8 in two sections (A and B). Here the inclusion has more the form of a broad belt than of a cord.

The cord-shaped inclusion may already develop buds which have a globular shape. The inclusion of the cell in Fig. 6 shows many of them so that the cord here begins to take the appearance of a rosary. Later there is the tendency for such buds to be pinched off. In Fig. 8A this can be seen on the bud (**b**) which is still joined to the main cord, but only by a thin bridge.

Relatively late, when compared to the development of the inclusions in the lymphocystis cells of the perches, fenestrations appear in the inclusion bodies of the Centrarchidae. One hole (**h**) can be seen in the cord-shaped inclusion of the sunfish cell in Fig. 7B. Fig. 8B shows the belt-shaped inclusion pierced by three holes. In the lymphocystis cells of perches fenestrations of the inclusions appear earlier, already when the inclusions have still the shape of a calotte, and the perforations soon become so numerous that the inclusion bodies can be described as coarse networks.

In the following stages of the growth period of the lymphocystis cells of the Centrarchidae the inclusion bodies continue to expand in the cytoplasm. A number of globular buds sprout into branches, and more perforations develop. The branches and fenestrations then increase in size. So the whole configuration of the inclusion body rather frequently assumes the aspect of a "mycelium," as demonstrated in Pl. II, Fig. 9, in a bluegill lymphocystis cell of 225 microns in diameter. The cell is shown at three levels of focus of the microscope (Figs. 9 A, B, and C). The inclusion body had expanded in the peripheral zone of the cytoplasm and embraces the nucleus (**n**) which shows here, by exception, not one but two nucleoli (**e**). The most parts of the inclusion are connected as branches of one large "mycelium." This is also true for most portions of the inclusion which appear in the optical section B as separated. The portions marked c^1 and c^2 represent the areas where the "mycelium" from the lower level (C) can be followed into the next higher section (B) and from there into the top section (A). On the other hand, the small piece (**s**) in B, which can still be recognized also in A, is definitely isolated from the "mycelium," probably as a bud which was pinched off.

Not always, however, in bluegill lymphocystis cells of this size does the inclusion body still represent a connected "mycelium." Sometimes the "mycelium" is already fractioned into several globular portions. Such a partition of the inclusion body into separated fractions is the rule in the stages of the further development (cells of diameters of 300-600 microns).

It may be that the fractioning of the inclusion into numerous round bodies occurs earlier in the lymphocystis cells of the common sunfish than of the bluegill. Thus Pl. III, Fig. 10, shows numerous inclusion portions scattered through the peripheral zone of the cytoplasm in a sunfish cell of 200 × 185 microns. Many of them, especially the round forms, have not only the appearance of separated bodies in the section but are definitely isolated, as has been determined by study of the complete series of sections through this cell.

No essential difference was seen in the morphology of the inclusions between common sunfish and bluegill on one hand, and other species of Centrarchidae on the other hand. The lymphocystis cells of all contained, in the advanced stages of development, numerous separate inclusions which apparently also originated by budding and fragmentation of the original inclusion body.

As I have mentioned in a short note in 1937, also in the *Lachnolaimus* lymphocystis cells the shape and the condition of the inclusions in certain stages indicate their multiplication by budding and fragmentation. Such secondary increase in number by partition must be distinguished from the primary development of numerous inclusion bodies which appear independently one after the other in young lymphocystis cells. I described primary development of numerous inclusions in 1921b for the lymphocystis cells of *Pleuronectes;* however, on the basis of my observations in Centrarchidae and *Lachnolaimus,* it seems to me probable that, in addition to the primary development of numerous inclusion bodies, there may also occur in *Pleuronectes* a secondary increase in number by budding.

With regard to the distribution of the inclusions within the cytoplasm it is characteristic that the development of the inclusion bodies in the lymphocystis cells of the Centrarchidae remains restricted in the advanced stages to the cortical zone of the cell. A large central zone of cytoplasm which surrounds the nucleus is unoccupied by them as Pl. II, Fig. 9B (bluegill cell), and Pl. III, Fig. 10 (sunfish cell) demonstrate. This distribution of the inclusions resembles the position of the inclusion bodies in the lymphocystis cells of *Aleutera* and *Angelichthys,* but represents a striking contrast to the conditions found in full-grown lympho-

cystis cells of *Pleuronectes* and *Stizostedion*. In large *Pleuronectes* lymphocystis cells the whole cell body is filled with relatively thick inclusion bodies. In *Stizostedion* lymphocystis cells likewise in the advanced stages not only the cortical zone but the whole cell body is interspersed with thin folds of a single inclusion body which has sprouted into a garland with an enormous surface increase.

FINER STRUCTURE AND STAINING REACTION OF THE INCLUSION BODIES.

In the early stages the inclusions are represented by small bodies which look homogeneous (cf. Figs. 1, 3, 4, and left inclusion in Fig. 2). They stain intensively with nuclear dyes such as hematoxylin or safranin. As soon as they have reached a diameter of more than four microns their structure becomes differentiated (Fig. 2, right inclusion). Small clear vacuoles appear which in the following growth period progressively split up the basophil substance. The expanding inclusion bodies (Figs. 5-8) consist of a basophil framework which is interspersed with a ground substance staining lightly with hematoxylin or safranin. The framework appears in optical sections as a network of meshes of various sizes (Pl. I, Figs. 5-8).

It is very probable that the smallest meshes represent optical sections of alveoles. The larger ones, examined by focusing up and down, seem to be the components of a three-dimensional lattice-like structure. They vary in shape and thickness and may appear as threads, bands or lamellae. In the thicker portions of this basophil lattice small vacuoles can often be recognized, indicating the continuation of a splitting of the basophil substance which, on the other hand, apparently continues to increase in mass. As a matter of fact, the expansion of the inclusion within the cytoplasm is accompanied by a continuous increase in the number of the lattice meshes.

The smallest meshes have a diameter of only 0.8 microns. The openings of the largest meshes may reach a diameter of five microns. Often the inclusions contain some larger central spaces surrounded by a peripheral layer of smaller meshes which may represent either finer lattice networks or the walls of small alveoles (cf. Pl. I, Figs. 5 and 7).

In the thicker portions of the inclusions, for instance in the globular buds, several layers of lattice networks joined with each other by connecting meshes can be followed by focusing up and down. On the other hand, the lattice may approach a two-dimensional structure where the inclusion has expanded into a thin plate.

In the drawings of the inclusions in Pl. 1, Figs. 5-8, representing preparations stained with hematoxylin or safranin, attention was given chiefly to the configuration of the basophil framework and the microscope focused correspondingly. The vacuoles and the matrix in the smaller meshes appear in the drawings very palely shaded. In some of the larger central spaces, as in Fig. 5 (g) and Fig. 7A, the ground substance can be recognized more distinctly.

Figs. 5-8 show the structure of the lattice as it appears in a magnification of 625× when examined with an oil immersion objective and a low power ocular. In higher magnification the meshes of the lattice do not always appear homogeneous but may show a pattern of fine spots varying in depth of stain. Under most favorable optical conditions (powerful lenses, thin sections, appropriate differentiation of the staining) very fine basophil granules can be demonstrated as components of the basophil substance of the lattice. I intend to describe these finest structures in detail in a separate paper with figures which will show the inclusion bodies in considerably higher magnifications than here in Plate I.

As in my previous studies on the lymphocystis cells of *Acerina* and *Pleuronectes* the Biondi staining method was applied in the two Centrarchidae for the finer analysis of the components of the inclusion bodies. In the Biondi method the basic dyestuff, methyl green, is used in combination with two acid dyes, acid fuchsin and orange G. In confirmation of my previous findings it has been observed that also in *Lepomis* the basophil framework of the inclusions is electively stained with methyl green, exactly like the basichromatin of the nuclei. This staining reaction corresponds very well with the result obtained by Jirovec with the Feulgen reaction. Jirovec described in 1932 that the framework of the lymphocystis inclusions in *Pleuronectes* gives an intensely positive Feulgen reaction. Thus it seems justified to say that the framework of the lymphocystis inclusions consists of a susbtance which in its staining and microchemical reaction resembles basichromatin.

The vacuoles and the ground substance in *Pleuronectes* lymphocystis cells stain pink with the acid fuchsin in the Biondi method. It was on the basis of such Biondi preparations that I described in 1921 the "ground substance" of the lymphocystis inclusions generally as acidophilic. In the two Centrarchidae the result of the Biondi staining in this respect has been different. The vacuoles and the ground substance between the smaller meshes of the lattice remained either unstained or were slightly stained with methyl green. Only in the larger central spaces of the inclusions the ground substance stained pink in the Biondi method

and thus showed an acidophil staining re-action as in *Pleuronectes*. The cytoplasm stained likewise pink in these preparations. Cytoplasm and acidophil ground substance further resemble each other in their struc-tural appearance. After fixation in Flem-ming's fluid or in a mixture of absolute al-cohol (9.5 parts) and acetic acid (0.5 parts) both show a finely granulated structure.

The inclusion bodies in larger lymphocystis cells (cells of more than 200 microns in diameter) show the basophil substance as well developed only in the cortical zone. In the central portions of the inclusions the lattice has disappeared to a large extent. The central zone of the inclusions now con-sists of large confluent spaces filled with acidophil ground substance into which in-complete lattice septa project from the cor-tex. Usually the cortex layer of the lattice is perforated at one area so that the acido-phil ground substance from the central zone blends into the surrounding cytoplasm. Therefore, an originally globe-shaped en-largement of the inclusion body may now resemble a bowl or a basket. Under these circumstances the possibility has to be con-sidered that the acidophilic ground sub-stance in the inclusions of the Centrarchi-dae may be cytoplasm which has penetrated into the inclusion. However, such an inter-pretation would not preclude the possibility that the "cytoplasm" within the central por-tions of the inclusions might have become modified by addition of particles originating in the basophil substance of the lattice.

ORIGIN OF THE INCLUSION BODIES.

The left inclusion body of the lymphocy-stis cell in Plate I, Fig. 2, represents a rather early stage of development. The di-ameter of the homogeneous corpuscle is less than one micron. Thus the corpuscle is much smaller than the nucleolus. It lies at some distance from the nucleus, is embedded in the cytoplasm and surrounded by the char-acteristic halo.

In *Acerina* and *Pleuronectes* I was able to follow the microscopic appearance of the inclusion bodies from the very beginning (Weissenberg, 1914, 1920, 1921b). They first become recognizable within the cyto-plasm as very tiny granules. In such an early phase they can be distinguished from the ordinary cytoplasmic granules only by the surrounding halo. In the experimental infection of *Acerina* many fibroblasts begin to hypertrophy in the early part of the sec-ond week. Several of these hypertrophying cells soon become transformed into lympho-cystis cells by the appearance of surround-ing glassy membranes. Then several days pass in which the lymphocystis cells in-crease in size but do not yet show inclusion bodies. It was not before the *Acerina* lym-phocystis cells had reached the end of the

second week in the infection experiments, that I was able to discover in their cyto-plasm the earliest stages of the inclusion bodies as those tiny basophil granules sur-rounded by halos.

Thus I did not observe in the develop-ment of the inclusion bodies any morpho-logical relationship whatsoever to compon-ents of the nucleus of the host cell. Later when the grown inclusion has the shape of a calotte and lies to one side of the nucleus, it is true that the inclusion body in its coarse appearance might show a certain sim-ilarity with the nucleus due to the basophil staining of its framework. But with higher magnification I never found it difficult to distinguish the inclusion body from the nu-cleus of the host cell because the nucleus of the lymphocystis cells always contains at least one large acidophil nucleolus[2]. Under these circumstances I cannot agree with the suggestion of Nigrelli and Smith who in their filefish paper (1939) have considered a nuclear origin of the inclusion body.

THE APPEARANCE OF VARIOUS STAGES OF LYMPHOCYSTIS CELLS IN TRANSMITTED AND REFLECTED LIGHT.

As briefly mentioned above, the transpar-ency of the lymphocystis cells of *Lepomis gibbosus* and *macrochirus* changes when the cells have reached the size of 200 microns. The smaller lymphocystis cells appear gray and transparent in reflected light and do not show a special color in transmitted light. In cells larger than 200 microns in diameter ·the cytoplasm shows a yellowish color in transmitted light in contrast to the nucleus and the inclusion bodies which appear color-less. Inspected with reflected light under the microscope or in low magnification these cells appear white.

It might be thought that the change in the transparency and color is caused by the gradual increase in mass of cytoplasmic particles interfering with the course of the light. But the fact that the white color seems to appear somewhat suddenly at a certain stage of growth, rather suggests some alteration in the composition of the cytoplasm. Coarse refractive structures which could produce the optical effect of the white color are only occasionally to be seen in these "white" lymphocystis cells. There-fore, the supposition of a change in the mic-rostructure of the cell at the size of about 200 microns is suggested.

Mitochondria, which I studied in the lym-phocystis cells of *Acerina* with special meth-ods (Weissenberg, 1921b), are not yet in-vestigated in the lymphocystis cells of Cen-trarchidae. Thus it remains unknown whether lymphocystis cells larger than 200

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[2]In *Pleuronectes* numerous nucleoli are formed in the nucleus of the lymphocystis cells during the growth period.

microns in diameter are different from smaller lymphocystis cells in the development and distribution of mitochondria or other minute cell structures which might be demonstrated only by application of special methods. In routine preparations of fixed material, however, not any difference could be seen between the structure of the cytoplasm of the smaller and that of the larger lymphocystis cells. During the whole period of growth the cytoplasm shows under high magnification a finely granulated structure.

At present no evidence has been established to show that the suggested change in the microstructure of the cell body may be caused by a distribution of refractive virus particles within the cytoplasm of the lymphocystis cell. As a working hypothesis, however, such a possibility seems to me worthy of consideration. I may refer in this connection to observations made in studies on parasitic protozoa. In microsporidial diseases of fish, for instance in the infection of *Gasterosteus aculeatus* by *Glugea anomala* (Weissenberg, 1913, 1921a), the hypertrophying host cells appear transparent as long as they contain only the chains of schizonts of the microsporidium which have no refractive envelopes. But as soon as spores are formed which reflect the light, the zone of the host cell containing the spores appears white in reflected light.

Comparative observations on half-grown lymphocystis cells of *Stizostedion* in fresh condition indicate that such optical phenomena are not restricted to the lymphocystis cells of the Centrarchidae. They can also be seen in the *Stizostedion* lymphocystis cells but are not so conspicuous there. *Stizostedion* cells, of about 400 microns in diameter, appear whitish-gray in reflected light and pale yellowish in transmitted light. In this comparison it must be taken into consideration that in *Stizostedion* lymphocystis cells of this size the inclusion networks are not restricted to the peripheral zone of the cell; moreover, the whole cytoplasm is here interspersed with folded festoons of the inclusion network. Therefore, the conditions underlying the optical appearance of the whole cell body must be somewhat different from those of the lymphocystis cells of the Centrarchidae.

DISCUSSION.

Hypertrophy of host cells produced by the stimulus of intracellular parasites has been observed in various branches of the animal kingdom. The parasites which provoke this striking reaction are many different microorganisms belonging to bacteria or lower fungi or various groups of protozoa. In fish is known the occurrence of an enormous hypertrophy of host cells invaded by microsporidia. I myself have studied the gigantic growth of ganglion cells of *Lophius* infected by the microsporidium *Nosema lophii* (Weissenberg, 1911). Another striking example is the infection of certain connective tissue cells of *Gasterosteus aculeatus* by the microsporidium *Glugea anomala*. The round cells invaded by the microsporidium have at first only a diameter of about eight microns. Through the stimulus of the multiplying and sprouting intracellular parasite the host cells can reach ultimate diameters of 3,000-4,000 microns (Weissenberg, 1921a and 1913). In many of these examples of intracellular parasitism the hypertrophy of the host cell is accompanied by a characteristic metamorphosis.

It was on the basis of such observations that I have interpreted from the beginning the lymphocystis cells as fish cells stimulated to their gigantic growth and characteristic metamorphosis by an intracellular parasite. However, no bacterium, fungus or protozoon could be discovered in the lymphocystis cells. On the other hand, the lymphocystis disease proved to be very infectious. Furthermore, the development of the conspicuous inclusion bodies was observed in the cytoplasm of the growing cells and it was discovered that these inclusions pass through a stage in which they closely resemble the compact stage of the Guarnieri bodies of the variola virus infection. Thus, in 1914 and 1921b, I arrived at the conclusion that the supposed intracellular parasite might belong to the tiny parasitic microorganisms which v. Prowazek had described as Chlamydozoa and which at present are listed as "viruses." Under the general name "viruses" are united various obligatory cell parasites of very small dimensions. They are either of submicroscopic size or, as in their largest types (for instance the viruses of variola, vaccinia, fowl-pox, psittacosis), at the border of microscopic visibility.

It is true that it is not yet determined by filtration or ultracentrifugation experiments how small the size of the infectious particles is which transmit the lymphocystis disease from one host to another. Nevertheless, the formation of the conspicuous cytoplasmic inclusion bodies which in their development resemble those of some other virus diseases indicates clearly that the infectious agent of the lymphocystis disease represents a virus.

I have already pointed out that the early stage of the compact lymphocystis inclusion bodies closely resembles the compact stage of Guarnieri bodies produced by the viruses of the mammalian pox group. Points of comparison are not only the shape of the bodies and the surrounding halo but also the basophil staining reaction.

In the following stage of the beginning differentiation numerous vacuoles are

formed in the lymphocystis inclusions so that the latter appear as alveolar structures. In this stage the lymphocystis inclusions resemble very much the vacuolated plaques which have been described by Rake and Jones (1942) in the development of the inclusions of lymphogranuloma venereum. Vacuolated inclusion bodies of a rather similar appearance are also described in certain plant virus diseases, *e.g.* by Kunkel in the cells of the sugar cane in the Fiji disease, as demonstrated in Pl. 3, Fig. 10, of Kunkel's chapter in Rivers, "Filterable Viruses".

In the course of the further differentiation of the lymphocystis inclusions the characteristic lattice framework is formed which shows a staining and microchemical reaction like basichromatin. It is of interest that in the differentiated Guarnieri bodies certain structures are described by Bland and Robinow (1939) which have a corresponding basophil staining reaction and also give a positive Feulgen reaction. These structures have the shape of lumps and rods and sometimes split into small basophil granules in advanced stages. This basophil material is evidently the same substance which von Prowazek had already described in 1907 and 1912 as the chromatoid component of the Guarnieri bodies.

It is true that in no other virus disease such a considerable growth of inclusion bodies is observed as in the lymphocystis disease. But evidently this outstanding phenomenon of the enormous growth of the lymphocystis inclusion bodies is closely connected with the gigantic hypertrophy of the host cell which in no other virus disease is observed hitherto in such a degree. It is of interest that a certain increase in size of the infected host cells also occurs in some other virus diseases, *e.g.* in fowl-pox, in the polyedral diseases of insects, in the mosaic disease of corn[3]. But the enlargement of the host cells observed in these virus diseases is insignificant in comparison with the enormous growth of the lymphocystis cells. Small fibroblasts after transformation into spherical lymphocystis cells reach diameters of about 600-660 microns in the Centrarchidae, of 1,200 microns in *Stizostedion*, of 2,000 microns in *Pleuronectes*. This gigantic hypertrophy of the lymphocystis cells is obviously the indispensable prerequisite to the enormous growth and spreading of the inclusion bodies.

Concerning the significance of the lymphocystis inclusion bodies I suggested in 1921 that they might be carriers of sprouting colonies of a tiny cell parasite. The size of the individual unit of this intracellular microorganism was supposed to be very small and perhaps beyond microscopic visibility.

My present view differs from this interpretation in certain details but corresponds

[3]cf. Goodpasture, 1928; Glaser, 1928; Kunkel, 1928.

to it in the general concept. The growing inclusions seem to me to represent the manifestation of the growth of an intracellular parasite of which the earliest stages as well as the final stages of transmission are of very tiny size. A number of facts support such a view. 1) The inclusion bodies grow more rapidly than other components of the hypertrophying host cell. Thus the inclusion bodies sprout out through the cytoplasm. In Percidae and Centrarchidae where, as a rule, only one inclusion body continues to grow, it develops into a network or a "mycelium" which soon embraces the nucleus and then extends through the cortical zone of the cell (Centrarchidae) or throughout the whole cell body (Percidae). The rate of its growth surpasses the pace of the hypertrophy of the other cell components for a long period. 2) After the metamorphosis and hypertrophy of the host cell has indicated its invasion by the virus, several days pass in which inclusion bodies are not yet recognizable within the cytoplasm. Then they become visible at first as tiny points surrounded by halos and continue to increase in size during the following days and weeks. 3) In the lymphocystis cells of Percidae (*Acerina* and *Stizostedion*) I observed that accessory inclusion bodies, as a rule, remain rudimentary. This fact can be compared to the poor development that colonies of bacteria or fungi may show under unfavorable conditions of nutrition. 4) The budding and sprouting of the inclusion bodies, as described in the present paper in *Lepomis* lymphocystis cells, strongly suggest that the increase in size of the inclusion resembles that of a living material capable of growth and self-propagation.

The general view that the sprouting of the lymphocystis inclusions represents the manifestation of the expansion of the cell parasite within the host cell is in accordance with the observations and conclusions of many investigators who have studied cytoplasmic inclusions in other virus diseases.

To cite only a few examples, I refer first to the development of the Negri bodies characteristic of the lyssa virus infection. Paul and Schweinburg (1926) and Schweinburg (1937) have stated that these inclusions appear at first as tiny points in the infected nerve cells and then grow gradually to become the Negri bodies. These authors have likewise interpreted this development as the expansion of a parasitic organism within the host cell.

In a number of other virus diseases tiny granules, the so-called elementary bodies, have been observed in the infected tissues. By careful investigations in several laboratories during the past 18 years ample evidence has been established to show that very probably the elementary bodies represent the infective units of the respective

viruses. The elementary bodies have in vaccinia[4] and in fowl-pox a size of about 0.25 microns, in psittacosis of about 0.3 microns, in lymphogranuloma venereum (Rake and Jones, 1942) a size of about 0.4 microns. To the cytoplasmic inclusions of these viruses the elementary bodies are correlated in various respects. First of all, it has been stated that the inclusion bodies disintegrate at the end of their growth period into a large number of elementary bodies. Secondly, the development of the inclusion bodies has been followed from early stages in which they represent small corpuscles of about 1 micron or still less in diameter. These "initial bodies" are supposed to be enlarged elementary bodies which after entering the host cell have been transformed into the anlagen of inclusion bodies. Whether the increase in size of the elementary body is due to growth or to the deposition of some coating material, possibly produced by the host cell, has been discussed by Bland and Robinow (1939) in their study of the development of the Guarnieri bodies of vaccinia. Thirdly, in psittacosis and lymphogranuloma venereum the growing inclusion bodies have been demonstrated to be composed of relatively large granules of about 1 micron in size. These large granules are embedded in a matrix which holds them together. The large granules have been interpreted as developmental stages of the virus (Bedson and Bland, 1932 and 1934; Bland and Canti, 1935; Rake and Jones, 1942). They are derived from the original elementary body as products of repeated divisions. The large granules in the inclusions of lymphogranuloma continue to grow into plaques up to 4 microns in diameter (Rake and Jones). Within these plaques then appear elementary bodies of the original size. In psittacosis the large granules have been described as undergoing progressive divisions by which they decrease in size. The end products of these divisions are again elementary bodies.

The Guarnieri bodies of vaccinia are evidently more complicated in their structure and development. In advanced stages of these inclusions elementary bodies were seen by Bland and Robinow. But these authors were unable to identify elementary bodies within the growing Guarnieri bodies. Nevertheless, Bland and Robinow consider it very probable that the Guarnieri body during its whole development contains elementary bodies enveloped in a matrix which is possibly produced by the host cell. Thus they interpret the Guarnieri bodies as colonies of the virus units and conclude that they represent an obligatory stage of the multiplication of the virus within the host cell.

The cited examples show that the interpretation of cytoplasmic inclusions as colonies of the virus or as carriers of multiplying stages of the virus is a familiar concept in the modern virus literature. It is of decisive importance that in fowl-pox conclusive evidence has been established to show that the inclusion bodies do contain the infective material. Woodruff and Goodpasture succeeded in 1929 in producing a typical fowl-pox lesion by transplantation of an isolated inclusion body into the skin of a hen.

It may be gathered from this review that the general interpretation of the lymphocystis inclusion bodies as the manifestation of the growth of the virus within the host cell is in harmony with the structural analysis and the dominant interpretation of cytoplasmic inclusions in a number of other virus diseases.

Any attempt to put the general idea of the association of the lymphocystis inclusion bodies with the growing virus into a precise form has to deal with an old problem. Does the whole inclusion body represent substance of the cell parasite or have two components to be distinguished in the inclusion body: 1) developmental stages of the cell parasite and 2) a surrounding substance produced by the host cell? The latter opinion was established in 1907 by von Prowazek who described cytoplasmic inclusion bodies in virus diseases as complex structures consisting of tiny microorganisms and of an enveloping substance produced by the host cell as a reaction product. Von Prowazek considered the formation of the reaction product under the stimulus of the cell parasite as so characteristic that he called the infectious agents of virus diseases "Chlamydozoa" which name means literally animals equipped with a mantle[5].

It was on the basis of von Prowazek's theory that in 1921 I interpreted the lymphocystis inclusion bodies likewise as complex structures. I supposed that they consist of colonies of a tiny microorganism which are interspersed with structures produced by the host cell as a reaction product. I suggested that the granular acidophil ground substance, as described in the inclusion bodies of *Pleuronectes* and *Acerina,* might represent the location of the virus colonies. I interpreted the chromatin framework of the lymphocystis inclusion bodies as a reaction product of the host cell which might serve a mechanical function by localizing the growth of the sprouting colonies within the cytoplasm.

At that time the acidophil ground substance was the only component of the lymphocystis inclusion bodies in which a gran-

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[4] The elementary bodies of vaccinia have been studied by Green, Anderson and Smadel by means of the electron microscope (1942). The electron micrographs in a magnification of 7,100 x 4 show the vaccinia particles as rectangular bodies with the shape of a brick and a structure which in several respects approaches that of bacteria.

[5] cf. Cowdry in Rivers "Filterable Viruses," pp. 114 and 115.

ular structure was observed. In the chromatin meshes of the lattice no finer structures were described besides the frequent appearance of small vacuoles. My recent studies of sections with powerful lenses have shown, however, that the chromatin meshes of the lattice not always appear homogeneous. They may show a pattern of fine spots varying in depth of staining, and, under favorable optical conditions, fine basophil granules can be demonstrated as components of the lattice framework.

Under these circumstances, the possibility gains weight that the chromatin lattice might consist of virus substance or might contain developmental stages of the virus. The fact that the substance of the lattice contains nucleoprotein, as proved by selective staining and the Feulgen reaction, would be in favor of such an interpretation. It has been shown by chemical analysis that certain plant viruses are large molecules of a nucleoprotein which contains yeast nucleic acid (cf. Stanley, 1938, 1940). The studies of Rivers and coworkers of the more complex chemical composition of the vaccinia virus have demonstrated that among its constituents is again a nucleoprotein. This nucleoprotein is of the thymonucleic acid type. The nucleoprotein in the lymphocystis lattice framework is likewise of the thymonucleic acid type, as proved by its positive Feulgen reaction (Jirovec, 1932). Hoagland, Smadel and Rivers have shown in 1940 that 5.0 per cent. of the elementary body of vaccinia is thymonucleic acid. This result of the chemical analysis is in accordance with the fact that elementary bodies of vaccinia, when resuspended in aqueous media, give a positive Feulgen reaction in the test tube (Bland and Robinow, 1939; Smadel, Lavin and Dubos, 1940). In sections through cells infected by the vaccinia virus an intensively positive Feulgen reaction of the substance of the smaller Guarnieri bodies during their compact stage has been stated by Bland and Robinow (1939). In the larger differentiated Guarnieri bodies the basophil lumps and rods, which I have above compared with the chromatin framework of the lymphocystis inclusion bodies, remain distinctly Feulgen positive (Bland and Robinow).

An interpretation of the chromatin lattice of the lymphocystis inclusions as consisting of the growing virus substance or as containing developmental stages of the virus would not exclude the possibility that end products of the multiplication of the virus might become accumulated in the ground substance of the inclusion bodies. Should such end products serving as stages of the transmission of the virus become further distributed throughout the cytoplasm of the larger lymphocystis cells, then an explanation for the optical phenomenon of the white color of these cells might be offered, provided that mature stages of the virus units are represented by refractive particles. The presumption of such an optical appearance of the infective units is supported by the fact that the elementary bodies of several viruses such as vaccinia have been indeed characterized as refractive particles. In the description of the inclusion bodies of inclusion blennorrhoea (Lindner, 1910) and of ectromelia (Barnard and Elford, 1931) the high refractility of the elementary bodies has been emphasized (cf. Findlay, 1938, p. 310, and Elford, 1938, p. 193). Furthermore, I refer to the macroscopic appearance of fowl-pox lesions. Goodpasture (1928) describes eruptions of fowl-pox on the mucosa surfaces as white, opaque spots.

Several of the problems touched upon in the above discussion can be solved only by further experimental work. In comparison to the conditions in other virus diseases it might seem that the large size of the lymphocystis cells and their inclusion bodies would present very favorable conditions for an experimental study of the properties of the inclusions and their constituents, with special reference to their infectivity. On the other hand, an essential impediment to the extension of the infection experiments with lymphocystis virus has hitherto been the difficulty of obtaining a sufficient supply, from noncontaminated waters, of highly susceptible fishes which can well withstand transportation and can easily be kept in laboratory aquaria.

In the experiments with the two Centrarchidae only some of these prerequisites were fulfilled. Under more favorable local conditions, Centrarchidae may present rather good prospects for further experimental work with lymphocystis virus although after my experience with *Lepomis gibbosus* and *macrochirus* it can hardly be hoped that it will be possible to keep a stock of lymphocystis-infected specimens permanently throughout the different seasons.

It would be very desirable for further experimental work if lymphocystis disease should be discovered in one of the many small "tropical aquarium fishes" which can easily be maintained in small aquaria. In any respect, it will be of interest to follow lymphocystis disease in still other fish groups, not only to obtain a more suitable object for further experimental work, but also to study still more varied manifestations of this virus disease so outstanding in its morphology.

RESULTS AND CONCLUSIONS.

1. Susceptibility to lymphocystis disease is widespread in the Centrarchidae and the disease is widely propagated in the United States.

2. Because lymphocystis epidemics have been observed in centrarchids which are not only game fish but are also of value as food fish, the disease is of some economic significance.

3. As a rule, the lymphocystis disease of *Lepomis gibbosus* and *macrochirus* heals by sloughing off the tumors about four weeks after the expiration of their growth period.

4. Experimental attempts to transmit lymphocystis disease from *Stizostedion* to *Lepomis* remained unsuccessful. The experiment, however, to transmit the disease from *Lepomis macrochirus* to *L. gibbosus* had a positive result.

5. When the lymphocystis cells of *L. macrochirus* and *gibbosus* in their growth period have reached about 200 microns in diameter, their appearance in reflected light changes from gray and transparent into white and opaque.

6. The cytoplasmic inclusion bodies in the lymphocystis cells show certain similarities to inclusion bodies in some other virus diseases with regard to their development and some of their structures. The enormous increase in size of the lymphocystis inclusion bodies is inseparably connected with the gigantic hypertrophy of the host cells which represents a hitherto unique phenomenon in the pathology of virus diseases.

7. A number of facts supports the view that the growing lymphocystis inclusions represent the manifestation of the growth of the virus organism within the host cell.

8. By the positive Feulgen reaction (Jirovec, 1932) and by selective staining with methyl green it is proved that the basophil framework of the lymphocystis inclusions contains nucleoprotein of the thymonucleic acid type.

9. The basophil framework inspected under high magnification does not always look homogeneous but sometimes shows a pattern of fine spots varying in depth of stain. Under favorable optical conditions fine basophil granules can be demonstrated as components of the framework.

10. In consideration of these structures and further of the fact that the framework contains nucleoprotein in its chemical composition, the suggestion is presented that the basophil substance of the lymphocystis inclusions either consists of virus substance or contains developmental stages of the virus.

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EXPLANATION OF THE PLATES.

PLATE I.

Figs. 1-8. Young lymphocystis cells of *Lepomis gibbosus* (common sunfish) and *macrochirus* (bluegill).
General labels: (**e**) nucleolus; (**m**) cell membrane; (**n**) nucleus; (**i**) inclusion body.

Fig. 1. Bluegill. Lymphocystis cell of 14 microns in diameter from a section. Fixation with Flemming's fluid; safranin-light green stain. × 1,000.

Fig. 2. Bluegill. Lymphocystis cell of 21 microns in diameter from a section. Fixation with Flemming's fluid; safranin-light green stain. × 625.

Fig. 3. Bluegill. Lymphocystis cell of 22 × 20 microns from a section. Fixation with Flemming's fluid; safranin-light green stain. × 1,000.

Fig. 4. Common sunfish. Lymphocystis cell of 22 × 19 microns from a whole mount preparation. Fixation with absolute alcohol 95 parts, glacial acetic acid 5 parts; Delafield's hematoxylin stain. × 625.

Fig. 5. Common sunfish. Section through a lymphocystis cell of 64 × 54 microns. Fixation with Flemming's fluid; safranin-light green stain. × 625.
(**g**) ground substance.

Fig. 6. Bluegill. Section through a lymphocystis cell of 80 × 64 microns. Fixation with Flemming's fluid; safranin-light green stain. × 625.
(**b**) buds of the inclusion; (**d**) portion of the inclusion which lies beneath the nucleus.

Fig. 7. Common sunfish. Two sections (**A**) and (**B**) through a lymphocystis cell of 66 × 61 microns. Fixation with Flemming's fluid; safranin-light green stain. × 625.
(**h**) fenestration of the inclusion body.

Fig. 8. Common sunfish. Two sections (**A**) and (**B**) through a lymphocystis cell of 72 × 54 microns. Fixation with Flemming's fluid; safranin-light green stain. × 625.
(**B**) bud of the inclusion connected with the main inclusion body by a thin bridge.

PLATE II.

Fig. 9. Bluegill lymphocystis cell, 225 microns in diameter, from a whole mount preparation. Drawings made by focusing: (**A**) through upper third of the cell; (**B**) through equator of the cell; (**C**) through lower third of the cell.
Acetic alcohol fixation (cf. Fig. 4); Delafield's hematoxylin stain. × 340.
(c^1) and (c^2) portions of the inclusion body which can be followed through the three adjustments as points of connexion; (**e**) nucleolus; (**i**) inclusion body; (**m**) cell membrane; (**n**) nucleus; (**s**) separated piece of the inclusion.

PLATE III.

Fig. 10. Common sunfish. Section through a lymphocystis cell of 200 × 185 microns. Fixation with Flemming's fluid; safranin-light green stain. × 340.
(**e**) nucleolus; (**i**) inclusion bodies; (**m**) cell membrane; (**n**) nucleus.

Fig. 11. Bluegill. Stage of the 21st day of experimental infection. Whole mount preparation of the tail fin border. Three weeks before the start of the infection experiment the margin of the fin was amputated. At the time of the experimental infection the regeneration of the fin border was in progress. The preparation shows the development of young lymphocystis cells within the regenerated tissues. The epithelium has been brushed off to a large extent.
Acetic alcohol fixation (cf. Fig. 4); Delafield's hematoxylin stain. × 35.
(**bo**) bony plates of fin rays below the level of the amputation; (**d**) debris of bony plates which indicate the level at which the amputation was performed six weeks ago; (**l**) young lymphocystis cells; (**p**) pigment cells; (**re**) regenerated tissue of the fin membrane; (**rf**) regenerating end portions of the fin rays in which bony plates have not yet developed.
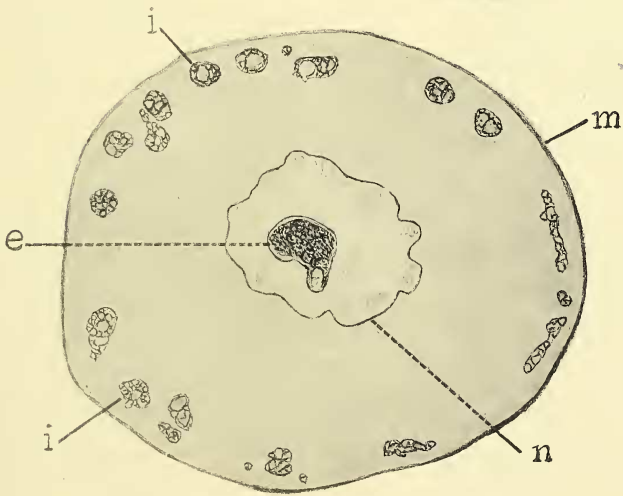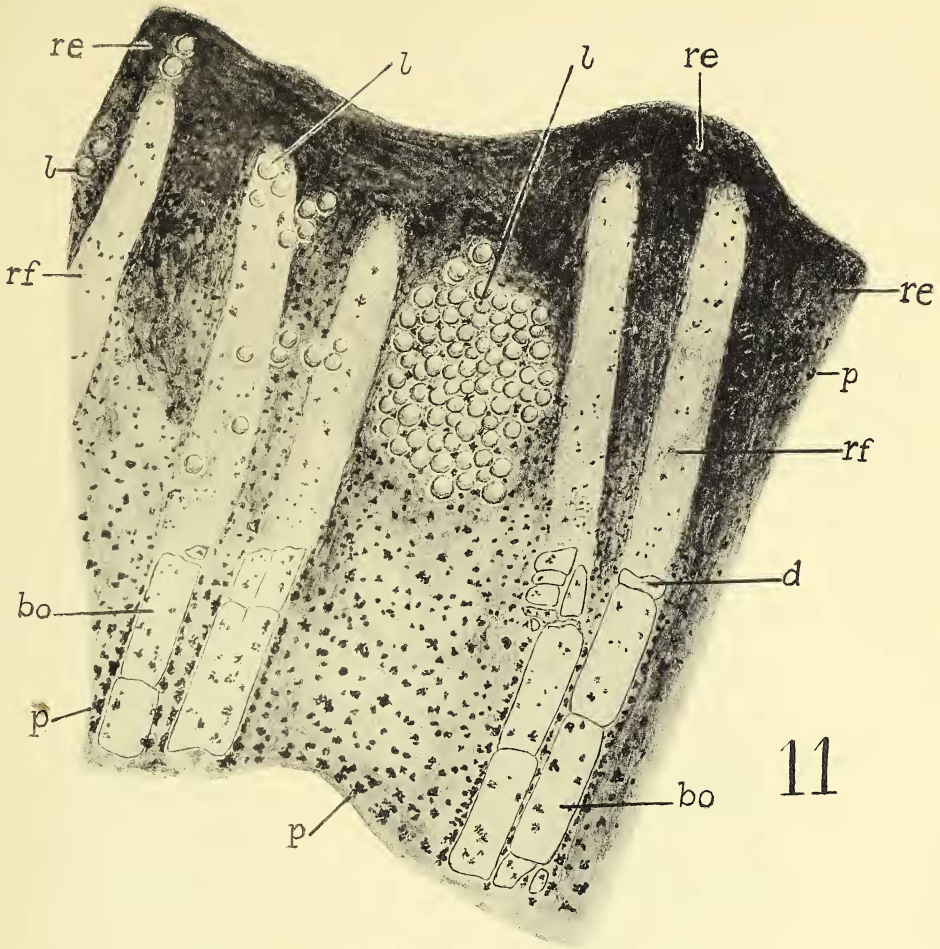
PLATE I.
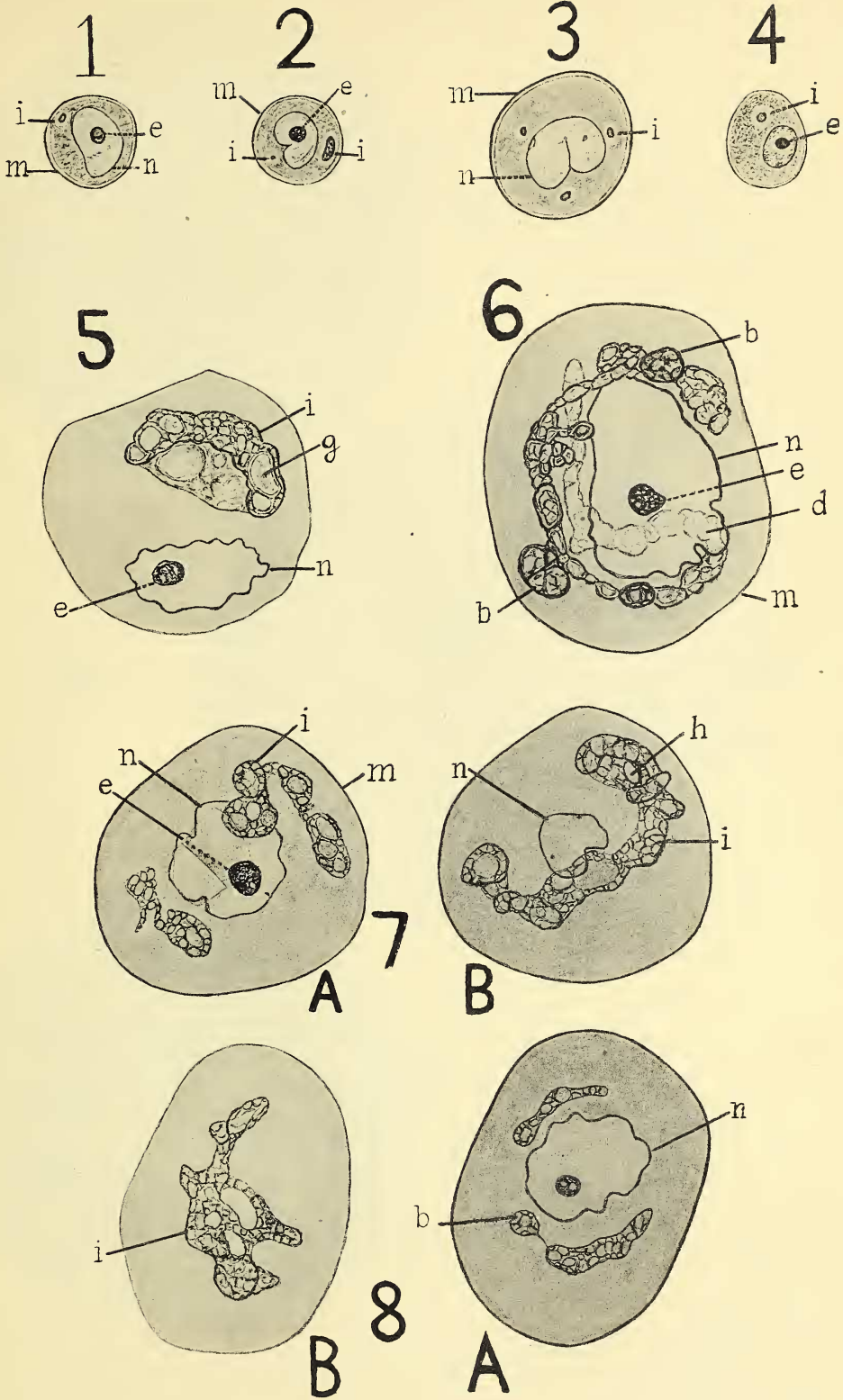
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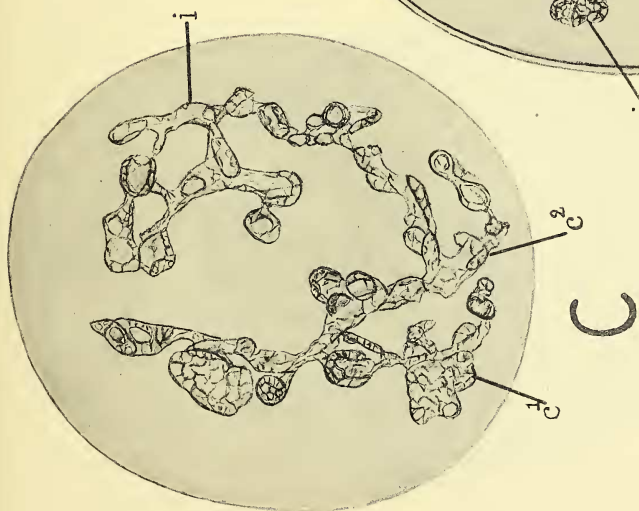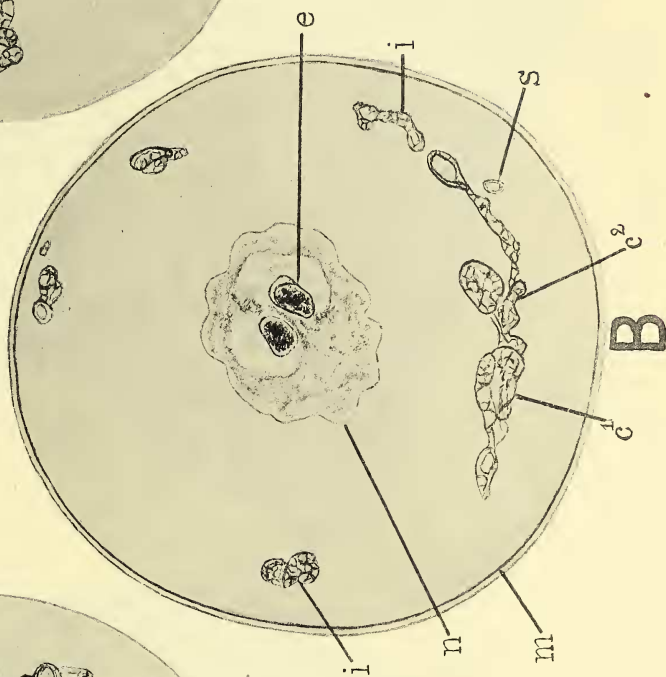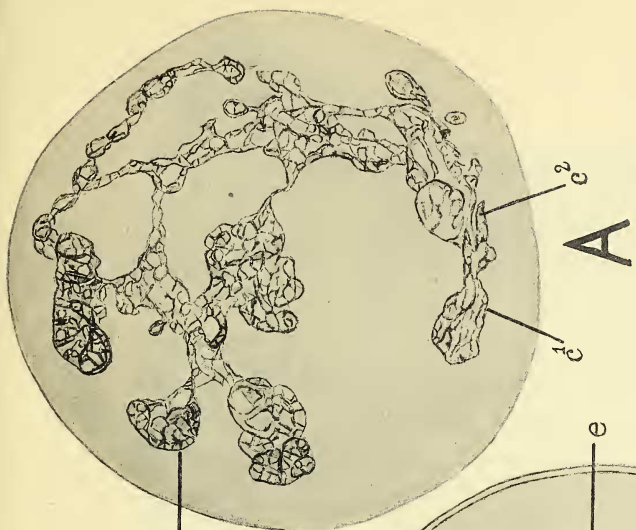
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